ANTI-AURORA-A MONOCLONAL ANTIBODY, METHOD FOR OBTAINING SAME, AND USES THEREOF FOR DIAGNOSING AND TREATING CANCERS

A subject of the present invention is a monoclonal antibody directed against aurora-A kinase of mammals, method for its obtention, as well as its uses in the context of the diagnosis or prognosis of cancers, and in pharmaceutical compositions for the treatment of cancers.

The aurora-A protein kinase is an oncogene, its overexpression in Rat-1 cells is sufficient to cause the appearance of a transformed phenotype and the implantation of these transformed cells into immunodeficient mice causes tumours to appear. (Bischoff et al., 1998; Zhou et al., 1998). The gene coding for this kinase is localized on the chromosome 20 at 20q13, amplicon frequently detected in numerous tumours (breast, colon, stomach cancers).

The overexpression of the aurora-A protein kinase has been observed in numerous tumours. Interestingly, the presence of this kinase in an abnormal quantity is not correlated to a proliferation detected by staining with a specific proliferation marker such as PCNA. Aurora-A is therefore a specific marker of the tumoral aspect of the cells (Tanaka et al., 1999; Takahashi et al., 2000).

Aurora-A belongs to a multigenic family of protein kinases called aurora, it comprises three members: aurora-A (described previously) aurora-B (Prigent et al., 1999) and aurora-C (Bernard et al., 1998). Although only aurora-A has a real oncogenic power the two other kinases have also been found overexpressed in the same tumours (Giet and Prigent, 1999).

The amplification of the gene coding for aurora-A is associated with the presence of an abnormally high activity of the protein kinase in these tumours. Moreover the ectopic overexpression of this kinase in cells in culture is sufficient to cause a transformed phenotype to appear, these cells transplanted into immunodeficient mice cause tumours to appear.

The overexpression of aurora-A kinase is very closely linked to the cancerous state of a cell. This overexpression of aurora-A kinase induces a polyploidy of the cells and causes an amplification of the centrosomes, two events which precede a poor prognosis for breast cancer for example.

It is therefore important to be able to precisely measure the expression of this kinase in cancerous pathologies, both at the level of the mRNA and the protein.

Now, the measurement of the expression of the aurora-A protein kinase depends entirely on the use of a good monoclonal antibody.

However, no sufficiently specific monoclonal antibody directed against the aurora-A protein kinase was able to be obtained until now, or is available commercially.

The subject of the present invention is to provide a reliable anti-aurora-A monoclonal antibody, which links with this protein with a sufficient specificity and sensitivity in order to envisage its use for purposes of experimental research, as well as in the field of diagnosis, prognosis and treatment of cancers.

The invention relates to an anti-aurora-A monoclonal antibody specifically recognizing the human and murine aurora-A kinase, and having the following properties:

* it can be fixed on the membranes containing the human or murine aurora-A protein,

* it allows detection, and, if appropriate, purification, of the human and murine aurora-A protein by immunoprecipitation,

* it allows the staining of biological tissues where the aurora-A protein is secreted and,

* it does not inhibit the enzymatic activity of the human and murine aurora-A protein,

said monoclonal antibody being as obtained by:

– five injections spread over fifteen days to mice of recombinant aurora-A protein kinase produced by *E. coli* bacteria transformed with a bacterial expression vector in the genome of which the human cDNA coding for aurora-A has been inserted, sacrificing said mice, and fusion between cells from the spleen of these mice and hamster cells immortalized in culture in order to obtain hybridomas,

– screening of the hybridomas producing an antibody capable of immunoprecipitating the recombinant protein used for the immunization of the mice during the preceding stage, and recovery of the positive hybridomas after this first screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein from an

extract of human HeLa cells in culture, and recovery of the positive hybridomas after this second screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of human cells in culture, and recovery of the positive hybridomas after this third screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein of mice from an extract of murine cells in culture, and recovery of the positive hybridomas after this fourth screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of murine cells in culture,

– recovery and purification by cloning a positive hybridoma after the previous screening stage, and producing a monoclonal antibody possessing all of the properties defined above.

Therefore, a subject of the invention is more particularly a monoclonal antibody as defined above, also called 35C1 antibody, said antibody being secreted by the hybridoma deposited on the 12th June 2003 at the Collection Nationale de Cultures de Microorganismes (CNCM) of the Institut Pasteur under the number I-3050.

A subject of the invention is also the use of a monoclonal antibody as defined above, and more particularly of the above-mentioned 35C1 antibody, for the implementation of an *in vitro* diagnostic or prognostic method for cancers in humans or animals.

A subject of the invention is more particularly the use of a monoclonal antibody as defined above, and more particularly the above-mentioned 35C1 antibody, for the implementation of an *in vitro* diagnostic or prognostic method for solid tumours, such as breast cancers, stomach cancers and colorectal cancers.

The invention also relates to the above-mentioned use of a monoclonal antibody as defined above, and more particularly of the above-mentioned 35C1 antibody, in combination with a cell proliferation marker, such as a marker of the PCNA protein (Tanaka et al., 1999; Takahashi et al., 2000).

A subject of the invention is also any *in vitro* diagnostic or prognostic method for cancers as defined above, in humans or animals, characterized in that it comprises:

– placing a monoclonal antibody as defined above, and more particularly the above-mentioned 35C1 antibody, in the presence of a biological sample taken from an individual, said antibody being if appropriate fixed on a solid support,

– the detection, and if appropriate the quantitation, of the aurora-A protein which may be present in the biological sample using marked reagents, in particular marked antibodies, recognizing either the monoclonal antibody linked to said aurora-A protein, or the aurora-A protein linked to said monoclonal antibody in the complexes formed during the preceding stage between the monoclonal antibody and the protein aurora-A which may be present in the biological sample, this, if necessary, after appropriate rinsing of the solid support.

Advantageously, in the context of the above-mentioned method, the determination of a quantity of aurora-A protein lower than or greater than a physiological threshold determined as a function of the biological sample, shows respectively a good or a poor prognosis for the diagnosed cancer.

A subject of the invention is also a kit for implementing a diagnostic method defined above, characterized in that it comprises:

– a monoclonal antibody as defined above, and more particularly the above-mentioned 35C1 antibody,

– if appropriate, a cell proliferation marker, such as a marker of the PCNA protein, in particular an anti-PCNA antibody.

The invention also relates to the use of a monoclonal antibody as defined above, and more particularly the above-mentioned 35C1 antibody, for the preparation of medicaments intended for the treatment of cancers, such as breast cancers, colorectal cancers and stomach cancers.

Therefore, a subject of the invention is more particularly any pharmaceutical composition, containing a monoclonal antibody as defined above, and more particularly the above-mentioned 35C1 antibody, in combination with a pharmaceutically acceptable vehicle.

A subject of the invention is also the use of a monoclonal antibody as defined above, and more particularly of the above-mentioned 35C1 antibody, for implementing a method for screening inhibitors of aurora-A kinase in which the lowering of the activity of this kinase is measured using said antibody.

A subject of the invention is more particularly any method for screening inhibitors of the aurora-A kinase characterized in that it comprises the following stages:

– the treatment of cells, such as lines derived from different cancers (breast, colon etc.), by the inhibitor tested,

– immunoprecipitation of the aurora-A protein kinase using a monoclonal antibody as defined above, and more particularly the above-mentioned 35C1 antibody, and measurement of the kinase activity, in particular according to the method described in paragraph 3. g) below.

The invention also relates to the method for the preparation of a monoclonal antibody as defined above, and more particularly the above-mentioned 35C1 antibody, characterized in that it comprises the following stages:

– five injections spread over fifteen days to mice of recombinant aurora-A protein kinase produced by *E. coli* bacteria transformed with a bacterial expression vector in the genome of which the human cDNA coding for aurora-A has been inserted, sacrificing said mice, and fusion between cells of the spleen of these mice and hamster cells immortalized in culture in order to obtain hybridomas,

– screening of the hybridomas producing an antibody capable of immunoprecipitating the recombinant protein used for the immunization of the mice during the preceding stage, and recovery of the positive hybridomas after this first screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein from an extract of human HeLa cells in culture, and recovery of the positive hybridomas after this second screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of human cells in culture, and recovery of the positive hybridomas after this third screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein of mice from an extract of murine cells in culture, and recovery of the positive hybridomas after this fourth screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of murine cells in culture,

– recovery and purification by cloning of a positive hybridoma after the preceding screening stage, and producing a monoclonal antibody possessing all of the properties defined above.

The invention is further illustrated with the detailed description of the 35C1 monoclonal antibody defined above and method for obtaining it.

The human cDNA coding for aurora-A (SEQ ID NO: 1) was inserted into a bacterial expression vector (pET29 Novagene).

The protein kinase was produced in BL21 (DE3)pLysS bacteria and purified by affinity chromatography on an Ni-NTA-agarose column (Qiagen).

The protein purified in the laboratory was then injected into mice (BALB/c).

After five injections spread over 15 days the mice were sacrificed and a fusion was carried out between cells of the spleen of the mouse and hamster cells immortalized in culture in order to obtain hybridomas.

The hybridomas obtained at a quantity of 960 were then tested for their capacity to produce an antibody which recognizes using Western blot the protein used for immunization.

The positive hybridomas after this first screening were then tested using Western blot for their ability to recognize the endogenous aurora-A protein from an extract of human HeLa cells in culture.

The positive hybridomas after this second screening were tested for their ability to recognize in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of human cells in culture.

The positive hybridomas after this third screening were then tested using Western blot for their ability to recognize the endogenous aurora-A protein of mice from an extract of murine cells in culture.

The positive hybridomas after this fourth screening were tested for their ability to recognize in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of murine cells in culture.

A hybridoma corresponding to all these criteria was retained and cloned in order to obtain a pure clone. This clone was named 35C1.

It secretes an anti-aurora-A monoclonal antibody which recognizes the human and murine aurora-A kinase.

This anti-aurora-A monoclonal antibody which specifically recognizes the human and murine aurora-A kinase has the following properties:

- it can be used in Western blot (indirect immunodetection of the protein on nitrocellulose or nylon membrane)
- it allows the protein in cells in culture to be located by indirect immunodetection
- it does not inhibit the enzymatic activity of the kinase in vitro
- it allows the aurora-A kinase from an acellular extract to be purified by immunoprecipitation
- because it does not inhibit the kinase activity of aurora-A it can be used to assay the kinase activity in protein extracts prepared from tissues which present pathologies.

1) Purification of the recombinant aurora-A protein

The cDNA coding for the human aurora-A kinase was cloned in the bacterial expression vector pET29 (supplier Novagen) which allows production of a recombinant protein containing 6 additional histidine residues. The BL21(DE3) pLysS strain of *E. coli* bacterium (supplier Promega) which is deficient in protease and which autolyzes by production of lysozyme after thawing (all these properties facilitate the purification of proteins) was used. The overexpression of the aurora-A(His)6-protein in the bacteria in the growth phase (OD_{600} = 0.6) is induced at 22°C by adding 1 mM IPTG (Isopropyl-β-D-thiogalactoside) over 4 hours. The bacteria are then lyzed at 4°C using in addition to their autolytic property, lysozyme and ultrasonics. The aurora-A-(His)6-protein is then purified by affinity chromatography on a nickel column Ni-NTA-agarose (supplier Qiagen). The protein is eluted with 250 mM imidazole following the Qiagen instructions. The purified protein is then passed over centricon YM-10 (supplier Millipore) in order to place it in a PBS solution (NaCl 136 mM, KCl 26 mM, Na_2HPO_4 2 mM, KH_2PO_4 2 mM, pH 7.2). Fractions of 15 µg of protein were prepared, lyophilized and stored at 4°C.

2) Immunization of the mouse

A BALB/c mouse was immunized by intraperitoneal route with 15 µg of recombinant aurora-A protein diluted in 50% Freund's complete adjuvant (supplier Sigma). The mouse was then injected with twice 15 µg of recombinant aurora-A protein diluted in 50% Freund's incomplete adjuvant with an interval of three weeks.

When anti-aurora-A antibodies were detected in the blood of the mouse, it was sacrificed and the spleen was removed. Cells in suspension were obtained from this spleen by homogenization with a Dounce.

These spleen cells were fused with SP2/O-Ag14 cells originating from a murine myeloma and obtained from the ECACC (Shulman et al., 1978). A fusion was carried out between 100.10^6 spleen cells and 20.10^6 SP2/O-Ag14 cells in 50% polyethylene glycol 1500 (supplier Roche) over 90 minutes at 37°C. The cells were then distributed in 10 x 96-well dishes containing a HAT selection medium (supplier Sigma Chemicals).

3) Screening of the hybridomas

a) ELISA

100 µl of PBS containing 4 µg/ml of recombinant aurora-A protein were deposited in each well of Elisa plates (96-well plates) and incubated for 36 hours at 4°C. After washing twice with PBS the wells are filled with PBS containing 3% BSA (Bovine Serum Albumin, supplier Sigma) and the plates are incubated overnight at 4°C. The next day 100 µl of each fusion supernatant is transferred into these 96-well plates containing recombinant aurora-A. The plates are incubated at ambient temperature for 2 hours. After washing twice with PBS/BSA, the plates are incubated with an anti-mouse antibody conjugated with phosphatase (Sigma Biochemical). The wells are then washed twice with PBS and once with an AP solution (100 mM Tris pH 9.5, 100 mM NaCl and 5 mM $MgCl_2$). The presence of a monoclonal antibody is detected after filling the wells with 50 µl of AP solution containing the synthetic phosphatase substrate (disodium 4-nitrophenyl phosphate hexahydrate salt) at 5 mg/ml (supplier Merck) and by the appearance of yellow staining in the well.

b) Western blot against recombinant protein

Ten 96-well plates (8x12) were analyzed by ELISA tests without producing very reproductive results. These plates were then tested by Western blot carried out in the following manner. The recombinant aurora-A protein was subjected to a polyacrylamide-SDS gel electrophoresis and transferred onto nitrocellulose membrane according to the technique described previously (Roghi et al., 1998). The membranes were cut in order to isolate the region corresponding to the locus to where the aurora-A protein migrated. The membrane ends were blocked by incubation in a TBST solution

(20 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% Tween 20) containing 5% milk for 2 hours at 4°C. Each membrane end was then incubated with the cell supernatants diluted to 1:100 in a TBST solution containing 2.5% milk for 1 hour at 4°C. The immunocomplexes were identified using either a second anti-mouse antibody conjugated with peroxidase or with phosphatase (supplier Sigma Chemicals) at the dilution recommended by the manufacturer. The development of the reaction was carried out by the chemiluminescence technique for the peroxidase (supplier Amersham Pharmacia Biotech) according to the instructions of the supplier or by colorimetry for the phosphatase using the two substrates NBT/BCIP (supplier Sigma Chemicals) according to the manufacturer's instructions.

The wells of each plate were grouped in pools of 8 corresponding to each column of each plate. The presence of monoclonals was analyzed in each pool by Western blot against the recombinant aurora-A protein. Of the 120 pools tested only 19 produced a positive response.

Each of the 8 wells corresponding to each positive pool was tested separately by the same Western blot technique with the aim of identifying which well(s) contain(s) antibodies. Figure 1 shows an example of results obtained with pool number 2, in this particular case only the wells A and B contained antibodies, the well having been retained.

Of the 120 pools tested only 19 were retained because they produced a very strong positive response. In these 19 pools only 23 wells contained antibodies directed against the recombinant aurora-A protein.

c) Western blot against the endogenous human aurora-A protein

The same Western blot technique was used this time to identify the supernatants capable of recognizing the aurora-A protein from all the proteins of a total acellular extract prepared from human cells in culture.

The cells chosen are HeLa cells. The extracts were prepared from culture dishes containing approximately 10^6 cells, the cells were lyzed in their dish with 1 ml of a so-called Laemmli solution corresponding to the solution deposited on polyacrylamide-SDS gel (Laemmli 1970), the solution was incubated for 10 minutes at 90°C, sonicated and centrifuged, 10 µl of the supernatant is deposited on the gel.

From the 23 supernatants which had been selected previously only 12 contained an antibody capable of recognizing a protein of 46 kD (expected size for aurora-A) by Western blots carried out on extracts of HeLa cells.

d) Immunofluorescence on human cells

An additional stage was introduced into the screening in order to select the antibodies which were capable of decorating the centrosome in human cells in culture. The choice of cells was for the cell line MCF7 which derives from a breast cancer because the aurora-A protein was reported to be overexpressed in these cells.

The technique used is indirect immunofluorescence. The cells are cultured on round glass slips in the 12-well dishes (supplier Corning Inc) for 48 hours. The slips are then washed with a PBS solution and the cells fixed with cold methanol (-20°C). The cells are then incubated for 30 minutes at ambient temperature in PBS containing 3% BSA. After washing three times with PBS the slips are incubated with the hybridoma supernatants diluted to 1:50 in PBS for 1 hour at ambient temperature. The cells are again washed three times with PBS and incubated at ambient temperature for 1 hour with a second anti-mouse antibody conjugated with fluorescein « FITC » (supplier Sigma Chemicals). The slips are washed three times with PBS and the cells are placed between the blade and slip in Mowiol containing antifading agent. The observations were carried out with a Leica DMRXA fluorescence microscope and the images taken with a black and white camera (COHU) were treated with Leica Qfish software.

Of the 12 supernatants retained previously only 4 contained antibodies capable of decorating the centrosomes and the poles of the mitotic spindle of the MCF7 cells. This localization corresponds exactly to that expected for aurora-A kinase.

e) Western blot against the endogenous murine aurora-A protein

With the aim of increasing the selectivity of the screening we tested the 4 supernatants against the orthologous protein of aurora-A in mice. A first screening was carried out by Western blot against acellular extracts of mice cells in culture, m-ICc12 cells. The acellular extracts were prepared as for the human cells and the Western blots were carried out in the same way as previously. Two of the supernatants were capable of recognizing a protein of 46 kD (size also expected for the murine aurora-A kinase).

f) Immunofluorescence on murine cells

We verified whether the two supernatants identified previously using Western blot were capable of decorating the centrosomes of cells of mice in culture. We chose the LLC1 cells because they present a very high mitotic index. Only one of the two antibodies was capable of localizing a protein in the centrosomes and at the poles of the mitotic spindle, localizations expected for the aurora-A protein kinase of mice.

g) Assay of the aurora-A kinase activity

Measurements of the aurora-A kinase activity were carried out in 20 µl of Tris-HCl 50 mM pH 7.5, NaCl 50 mM, DTT 1 mM, $MgCl_2$ 10 mM, and ATP 10 µM including 1 µCi of [γ-^{32}P] ATP 3000 Ci/mmole (supplier Amersham Pharmacia Biotech) containing 4 µg myelin basic protein (MBP) for Figure 5 (supplier Sigma Chemicals) or 10 µl of an extract of bacteria having produced the GST-H3 protein for Figure 6. The reactions are incubated at 37°C for 10 minutes. 10 µl of the reaction are analyzed either during counting (Figure 5) or after migration on polyacrylamide-SDS gel, dried and examined by autoradiography (Figure 6).

h) Cloning of the selected monoclonal

The supernatant that we have selected contained a heterogeneous mixture of cells obtained after fusion. We have subcultured these cells carrying out a limited dilution and obtained 20 clones. The supernatant of these 20 clones was tested using Western blot against the recombinant aurora-A protein, 8 produced a positive response. These 8 supernatants were tested on extracts of human HeLa cells, of murine m-ICc12 cells. Only two supernatants were retained.

These two supernatants were recloned again by limited dilution and retested as previously. The aim of this last cloning was to select a clone which maintained a level of antibody production which can be reproduced after subculture.

Only one of the two clones proved to be stable, it was named 35C1 and retained for storage and production of monoclonal.

i) Properties of the 35C1 monoclonal (see figures)

The antibody specifically recognizes the human and murine aurora-A protein kinase using Western blot in total acellular extracts (Figure 1).

It localizes the aurora-A protein kinase in humans cells and in murine cells in culture (Figure 3).

It immunoprecipitates the aurora-A protein from extracts of human MCF7 cells (Figure 4).

It does not inhibit the kinase activity of aurora-A (Figure 5).

It therefore allows the immunoprecipitation of the aurora-A protein and measurement of its kinase activity while it is still combined with the antibody (Figure 6)

These properties of the 35C1 monoclonal make it a tool of choice for the study of the aurora-A protein kinase.

It can be used in diagnostic and prognostic methods for solid tumours. The level of expression of the mRNA coding for the aurora-A protein is closely correlated with the genetic instability of the breast cancer cells and with a high-grade tumour (Miyoshi et al. 2001). This was very clearly established for breast cancer. On the other hand because of the absence of sufficiently specific monoclonal antibodies, this correlation between the quantity of aurora-A mRNA and the grade of the cancer has not yet been able to be verified at the protein level. The anti-aurora-A 35C1 monoclonal will allow this type of measurements. It allows on the one hand measurement of the quantity of aurora-A protein (Western blot and immunohistochemistry) and on the other hand measurement of the aurora-A activity (immunoprecipitation) in tumours, and thus determination of the threshold of the quantity of aurora-A below which and above which the prognosis for a determined cancer is respectively good or poor.

Moreover, the 35C1 antibody allows testing of the effectiveness of inhibitors of the in vivo aurora-A kinase activity. The aurora-A protein kinase is immunoprecipitated from HeLa cells for example previously treated by the inhibitor and its activity measured *in vivo*. This allows among other things the evaluation of the stability of the inhibitors in vivo.

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Legend of the figures

Legend of Figure 1: Scanning of the hybridomas by Western blot. The purified recombinant aurora-A protein was deposited on polyacrylamide-SDS gel and transferred onto a nitrocellulose membrane. The membrane was stained poppy red and the band corresponding to aurora-A was cut out. Each panel corresponds to a piece of membrane with aurora-A. After fusion the cells were distributed in 96-well dishes. In order to screen the presence of anti-aurora-A monoclonals of the aliquots of the supernatants, wells of each column are grouped in pools, this being done for each dish. Each pool is then tested using Western blot right-hand column from 1 to 12. When a pool is considered to be positive, here the pool number 1, the supernatants of each well which constitute this pool (from A to H) are retested individually. In this specific case the wells A and B contained antibodies, but only well B was retained.

Legend Figure 2: Western blot. The total acellular extracts are separated on polyacrylamide SDS gel and the gel is transferred onto nitrocellulose membrane. Well 1 does not contain extract and well 2 contains 10 µl of extract (corresponding to 10^6 cells per ml). The antibody is used at a dilution of 1/100. Only the aurora-A protein of 46 kD is detected.

Legend Figure 3: Indirect immunodetection of aurora-A in human and murine cells. The human cells are MCF7 and the murine cells are LLC1. In immunofluorescence DNA is detected by staining DAPI (blue), γ-tubulin (red) and aurora-A (green).

Legend Figure 4: Immunoprecipitation of aurora-A. The protein is immunoprecipitated by the 35C1 antibody conjugated with the A-Sepharose protein. The immunoprecipitates are separated on a polyacrylamide-SDS gel, the gel is transferred and the immunocomplexes revealed with the 35C1 monoclonal. Well 1: the 35C1 antibody only; well 2: immunoprecipitation carried out with the A-Sepharose protein only; well 3: immunoprecipitation carried out with the 35C1 monoclonal antibody; well 4: immunoprecipitation carried out with an antibody prepared in the laboratory.

Legend Figure 5: Activity of the purified human recombinant aurora-A kinase measured in the presence of the 35C1 monoclonal antibody. The 1C1 antibody directed against the aurora-A protein of the xenopus genus and which does not cross with the human protein is used as control. The kinase activity is measured using MBP (Myelin Basic Protein) as substrate.

Legend Figure 6: Activity of the endogenous aurora-A protein immunoprecipitated by the 35C1 antibody fixed on protein beads A-Dynabeads. The kinase activity is measured on a substrate comprising only one serine which can be phosphorylated. It is a GST construction in fusion with the tail of the H3 histone (with serine 10). A control substrate is also used where the serine 10 is replaced by an alanine. Wells 1, 4 and 7 contain purified recombinant aurora-A are used. Wells 2, 5 and 8 contain immunoprecipitated recombinant aurora-A and are fixed to the antibody and to the A-Sepharose protein. Wells 3 and 6 do not contain kinase. Wells 3, 4 and 5 contain the phosphorylatable substrate GST-H3(S) and wells 6, 7 and 8 the non-phosphorylatable GST-H3(S/A) substrate for the kinases.